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Replacement Ion Chromatography (RIC) is a new detection scheme for ion chromatography with the potential of very high sensitivity and universal application. In this first example of RIC, separated sample cations are stoichiometrically replaced by a photometrically sensitive cation. Following replacement, the RIC effluent is nebulized into a flame where emission from the photometric species is generated and measured by a filter photometer. The conditions for useful operation of the new RIC instrument are presented and applications are provided of sensitive and efficient sample replacement. The limitations and capabilities of the technique are critically appraised and possible extensions considered.

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REPLACEMENT ION CHROMATOGRAPHY
WITH FLAME PHOTOMETRIC DETECTION
I. CATIONS

by

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27 September 1982

The separation of aqueous ions by ion-exchange chromatography is striaght-forward and can be performed routinely. Unfortunately, the sensitive detection of the separated ions, specifically inorganic ions, is not as simple as their separation. Recently, suitable detection schemes to measure the conductivity of flowing chromatographic eluents have been developed [1-4]. These detectors allow convenient monitoring of low-pK_d-value ions that cannot be measured by optical absorption or electrochemical means. The technique, termed ion chromatography, has adequate sensitivity for many applications [5] and is currently a rapidly growing area of research.

One problem associated with the use of conductivity detectors for ion chromatography (IC) is the high conductance of solvents needed to achieve separation of the sample ions. Two approaches have been successfully employed to cope with this difficulty; the background conductance of the solvent can be suppressed chemically or electronically.

Chemical suppression of conductance was developed first [1]. In this approach, a column is placed in series with the analytical column. This "suppressor" column contains an ion-exchange resin capable of reducing (by acid-base neutralization) the conductance of the solvent. Sample ion conductivity is then measured against a much lower background conductance. The use of two columns in this approach has several minor drawbacks. Separation is slowed, chromatographic resolution is reduced, sensitivity is lowered slightly, and suppressor column regeneration can interrupt determinations. However, these problems can be overcome or tolerated and conveniently used systems are now commercially available.

Electronic suppression of solvent conductance requires only one column and offers high-speed separation and better chromatographic resolution than the two-column systems. It is also inherently more simple and reliable, but

requires the measurement of small conductance changes in the presence of the relatively high solvent conductance. Appropriately, the emphasis in this area of single-column technology is in the development of low-capacity ion-exchange resins for use with low-conductance solvents [2-4]. Ion separation units based on this latter scheme are also commercially available.

Conductivity measurements themselves have several drawbacks, the most important of which are limited sensitivity and an intolerance to temperature changes. Conductivity values can vary by as much as 0.5-3%/°C [6]. Accordingly, conductivity detectors must be carefully thermostated or must use reference cells for temperature compensation [7].

Another type of single-column IC technology has recently been reported [8]. Indirect photometric chromatography measures the transparent sample ion concentration by monitoring the changes in absorbance that the solute causes in a photometrically absorbing solvent. This alternative technique offers high-speed separations and good resolution. Under carefully selected conditions, sensitive measurement of ions is possible; however, determinations performed in this manner are restricted to solvent systems that have adequate absorbances at chromatographically useful concentrations. Importantly, weakly conducting but separable ions can be determined with this detector and its temperature dependence is negligible.

The purpose of the present paper is to describe and evaluate a new detector for ion chromatography which exhibits higher sensitivity than the designs described above, yet is universally applicable to all ions separable by IC. Importantly, the scheme is useful for the detection of both conducting and relatively nonconducting anions or cations and is almost completely temperature insensitive. This new technique, which we term Replacement

Ion Chromatography (RIC) is introduced here as a method for cation measurement and employs flame photometric detection. However, alternative applications and experimental arrangements are possible and will be suggested.

RIC is a concept where sample ions are first separated chromatographically and are then stoichiometrically replaced in the eluent by an ion that can be detected more sensitively than the sample ion itself. Nadmeyer, Lamb and coworkers [9,10] have replaced divalent sample cations with H^+ to improve conductivity measurements in systems using Pb^{+2} eluents with precipitation suppressors. Instabilities caused by pH effects were also minimized. In the example of cation chromatography presented here, sample cations are stoichiometrically replaced by Li^+. The eluent is then directed to a flame photometer dedicated to lithium measurement. Chromatograms are therefore simply a plot of lithium concentration as a function of time. Qualitative identification of individual ions is made by means of their retention times, the same procedure used with conductivity (or other nonspecific) detectors. Either peak height or peak area can be used for quantitation.

In this first example of RIC with flame photometric detection, several distinct advantages are realized. First, lithium (and therefore sample cations) can be measured very sensitively; detection limits for lithium in flame emission photometers are well below the part-per-billion level [11]. The reasons for this high sensitivity are the very low spectral background from hydrogen-air flames at the Li 670 nm spectral line and the small spin-orbit splitting in the $2^2P_{3/2,1/2}-2^2S_{1/2}$ transitions in lithium [12,13]. Second, calibration curves are linear over several orders of magnitude of concentration. Third, the detector is relatively insensitive to fluctuations in ambient temperature; and finally it is amenable to the quantitation of nonconducting or weakly conducting ions.

In its present embodiment, RIC is implemented by placing an ion-exchange column between the separator (analytical) column and the flame photometric detector. This "replacement" column contains a pretreated ion-exchange resin with the same polarity as the separator column, in a specific ionic form. The ions (here, Li^+) bound to the replacement column have a relatively low affinity for the resin compared to sample ions. Therefore, as sample ions enter the replacement column, they become bound to the resin and displace the replacement ions (Li^+) into the eluent. Charge balance assures that a stoichiometric exchange occurs, producing reliable, quantitative measurements. A detector for RIC can therefore be dedicated to the monitoring of a single ionic species: the replacement ion.

A detector dedicated to the measurement of a single ionic species can be optimized for that function and should be an inherently sensitive device, especially when the detector is mass sensitive, as is the flame photometer. Moreover, only a single ionic species is needed for calibration, assuming unity replacement efficiency. Of course, peak areas would be utilized in single-ion calibration.

EXPERIMENTAL

A modular liquid chromatograph was constructed and coupled to a laboratory-assembled flame photometer. The liquid chromatograph consisted of a high-pressure pump (Milton Roy Co. mini Pump, Riviera Beach, FL), a four-port injector valve (Altex model 210, Berkeley, CA) and a 0.25-mL sample loop. A commercial 25-cm cation column (Wescan Instruments, Santa Clara, CA) was used to separate sample cations. Suppressor and replacement columns were constructed from 0.25-in. o.d. stainless steel tubing. Bio-Rad AG1-X8 anion exchange resin was used to pack the suppressor columns whereas a Dowex

50W-X8 cation exchanger was used in the replacement columns. Columns of different lengths and internal diameters and containing resin of different particle sizes were constructed for testing. Dry-resin packing procedures produced columns that were superior to those which were slurry-packed. Modular flexibility was provided by appropriate low-dead-volume chromatographic fittings (Swagelock model SS-400-6-1LV, Crawford Fitting Co., Niagara Falls, Ontario).

The flame photometer consisted of a total-consumption air-hydrogen burner (Beckman Instruments, Inc., Fullerton, CA), a 50-cm focal-length, 40-mm diameter lens (Ealing Optics, South Natick, MA), a small monochromator, operated with a spectral bandpass of 0.67 nm (JY H-20, Instruments SA, Inc., Metuchen, NJ) and a red-sensitive photomultiplier tube (model R446, Hamamatsu Corp., Middlesex, NJ). The output photocurrent was amplified by an electrometer (model 610 A, Keithley Instruments, Inc., Cleveland, OH), filtered by a passive RC filter with a one-second time constant and recorded on a strip-chart recorder (model SR-204, Heath Co., Benton Harbor, MI).

In this work, the suppressor column was prepared in the hydroxide form whereas the replacement column was converted after packing to the lithium form. These two columns were simultaneous regenerated, when necessary, with 1 $\underline{M}$ LiOH, and were subsequently rinsed with deionized water.

RESULTS AND DISCUSSION

Table I lists the order of relative affinity of various cations for the cation exchange resin [14] used here in the replacement step of RIC; those cations with higher affinity values will displace those of lower affinity. Because lithium has the lowest affinity for the resin, it can serve as a

universal replacement cation. Conveniently, Li is also one of the most sensitive elements for determination by flame emission photometry, making it an ideal choice for the replacement ion in RIC. Figure 1 illustrates the absolute sensitivity achievable with the flame photometric detector in the absence of significant degradation of chromatographic resolution. A detection limit of 0.1 parts-per-billion (14 nM) is calculated. Clearly, this method of detection shows excellent promise as a sensitive detector for ion chromatography.

In addition, the analytical working curves produced with this method were nearly identical to those of conventional flame photometers for alkali metals [12]. At very low Li concentrations ($\sim 10^{-7}$ M), the classical ionization upward curvature is found, while at high Li concentrations ($> 10^{-3}$ M), self-absorption is observed. The net result is a sigmoidal-shaped working curve with its linear region between 10^{-6} and 10^{-3} M.

Of course, under conditions of high concentration, cations with lesser affinity for a resin can displace those with greater affinity, a property exploited during column regeneration. In addition, an ion of lower affinity than others serves as a convenient eluting reagent for those others, the reason that H^+ is often used to separate monovalent cations. Unfortunately, hydrogen ion used as an eluent would displace lithium quantitatively from the resin in the replacement column. To lower the concentration of H^+, a basic suppressor column is used in the present RIC procedure to raise the pH of the acidic eluent before it enters the replacement column. Without this suppressor column, lithium would be continuously displaced from the replacement column (Li bleed), requiring frequent regeneration of the column, producing an inordinately high flame photometric background and reducing

measurement sensitivity and precision. Incidentally, the lithium signal level shown in Fig. 1 is the theoretical level of lithium bleed from the three-column system if the suppressor column raised the pH of the acidic eluent to pH 7 and if no residual cation concentration in the system were above 10^{-7} $\underline{M}$.

Repeated injection of 0.25-mL samples of monovalent cations (cf. Fig. 2) produced RIC peak areas that were reproducible to within 3% relative standard deviation for concentrations above 0.1 m$\underline{M}$. Unfortunately, results obtained below 0.1 m$\underline{M}$ (0.05-0.01 m$\underline{M}$) were irreproducible and limited sensitivity to above 0.01 m$\underline{M}$. The cause of this loss in precision was traced to the suppressor column, where sample cations were apparently being retained. Interestingly, the Li$^+$ counter ion of the hydroxide solution used for suppressor regeneration was also retained, even after rinsing with copious amounts of deionized water. These retained cations bled slowly from the suppressor column and produced a substantial Li background signal, thereby further degrading detection of sample-generated peaks.

With a replacement column installed, a chromatogram of monovalent cations such as that shown in Fig. 2 can be generated. In Fig. 2, a slight deterioration of chromatographic resolution can be noted because of dead volume produced by the suppressor and replacement columns and fittings that connect them. Clearly, smaller particle sizes in these two post-columns would improve resolution. Importantly, the individual peak areas in Fig. 2 are equivalent to within the accuracy of manual measurement, reflecting the equimolar cation concentration in the original sample and verifying that the replacement phenomenon proceeds quantitatively. Peak area calibration for all ions should therefore be possible by use of a series of standards containing a single ion (e.g. Li$^+$).

By isolating each of the columns, it was found that most of the degradation of chromatographic resolution caused by the three-column system was associated with the replacement column. Improved replacement columns are now being tested to reduce this loss of resolution. No modifications of the total-consumption burner were made to decrease the dead volume of the detector. However, chromatograms obtained with this burner provided superior resolution to that obtained earlier with a premix burner-flame system.

Sensitivity provided by the present instrument, like precision, is linked to Li^+ bleed from the three-column system. In most experiments, overall bleed produced a Li^+ concentration in the replacement-column effluent of approximately 0.010-0.020 $m\underline{M}$. Eluent fractions collected at various points in the system indicated that the 12.5-cm suppressor column changed the pH of the 3.14 $m\underline{M}$ nitric acid solvent from 2.5 (entering) to 5.5 (leaving). However, the replacement column then raised the pH to 6.5, indicating that hydrogen ion attack of the replacement column was responsible for some background Li bleed. A longer suppressor column (25 cm) improved this undesirable situation, but exacerbated the problem of physical loss of sample cations in the suppressor column. Flame photometric examination of the fractions collected for pH determination showed that the majority of the Li present in the eluent originated in the suppressor column, and was a reminant of the regeneration process. Other bases, NaOH and KOH, used for regeneration exhibited the same behavior and produced subsequent Li bleed from the replacement column.

Importantly, earlier experimental results obtained with a Li 670 nm interference filter (bandpass 8 nm) are similar to those presented here. However, the narrow spectral bandpass of the monochromator rejects unwanted light from room illumination.

It should be possible also to separate and quantitate divalent cations by RIC. However, if divalent cations are to be determined and if flame photometric detection is employed, it becomes critical that the suppressor column be extremely efficient in removing eluent cations from the system. The relatively high affinity of divalent cations for the resin in the replacement column would otherwise lead to an extremely high lithium background and would make sensitive measurements impossible.

Unfortunately, hydroxide suppressor columns used in conjunction ith ethylenediamine or copper (II) eluents have not been very successf v applied to the separation and measurement of divalent cations such the alkaline earths. Exposure to basic conditions (pH 10.5) in the su; or column can increase sample losses there. Also, the relatively large dissociation constant for ethylenediamine ($K_{B_1} = 8.5 \times 10^{-4}$) prohibits complete charge neutralization, even at high (> 11) pH. Without charge neutralization, the replacement column resin exhibits severe Li bleed and undergoes irreversible attack by $NH_2C_2H_4NH_3^+$.

Lower-pH suppressor columns have been used in precipitation reactions with Pb (II) eluents [9,10] with good success for divalent cation separations. However, treatment of spent suppressor columns is cumbersome with this procedure. In preliminary experiments, we have had the best success with a hydroxide-suppressed Cu (II) system. Mg^{+2} and Ca^{+2} are easily separated and both replace Li extremely well. Unfortunately, the precipitation of $Cu(OH)_2$ involved in this procedure quickly results in high column back-pressure, lengthens elution time and severely broadens chromatographic peaks. In addition, the precipitation-suppression reaction was not completely efficient; a 25-cm suppressor column could change the Cu^{+2} concentration

in the eluent only from 4.0 m$\underline{M}$ (entering) to about 0.4 m$\underline{M}$ (leaving). This high residual Cu^{+2} concentration produced, of course, an unwanted Li bleed from the replacement column. Capture of sample cations by the suppressor column was an even greater problem during divalent cation separation than in the monovalent cation experiments. Sample precipitation (as hydroxides) is common, as is physical occlusion of sample cations in the eluent precipitate. Regeneration of the suppressor and replacement columns was also more time-consuming because of the type and severity of these reactions involving the Cu^{+2}. Alternative approaches to solving this problem are currently being investigated in our laboratory.

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TABLE I

Relative affinities of various cations for the ion-exchange resin used in replacement column [14]

| Ion	Relative Selectivity for AG 50W-X8
Li^+	0.85
H^+	1.0
Na^+	1.5
NH_4	1.95
K^+	2.5
Mg^{+2}	2.5
Cu^{+2}	2.9
Ca^{+2}	2.9

FIGURE CAPTIONS

Figure 1. Flame photometric detection of 10^{-7} $\underline{M}$ lithium. Flame coupled directly to separation column. Eluent: HNO_3; pH 2.5; 1.2 mL/min. Detection limit calculated as 14 $n\underline{M}$ at a signal-to-background-noise ratio of 2.

Figure 2. Detection of monovalent cations (1 mM each) by RIC. Eluent: HNO_3; pH 2.5; 1.2 mL/min; suppressor column: 12.5 cm x 3 mm i.d., 100-200 mesh particle size anion exchange resin; replacement column: 12.5 cm x 3 mm i.d., 20-50 mesh particle size cation exchange resin.

Li Response

Minutes

0   5
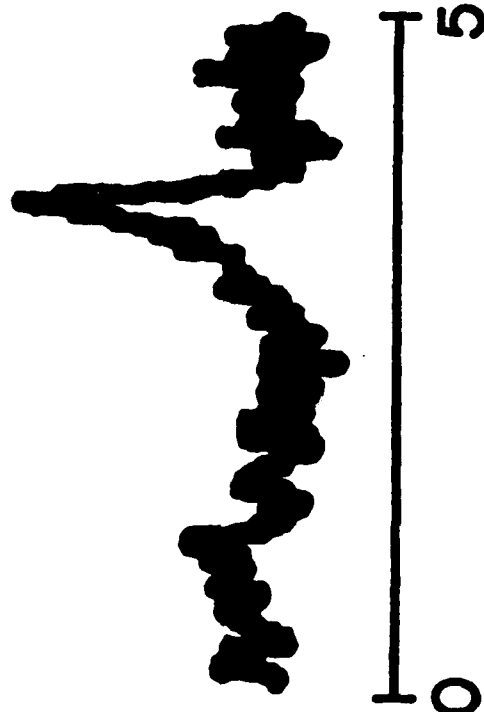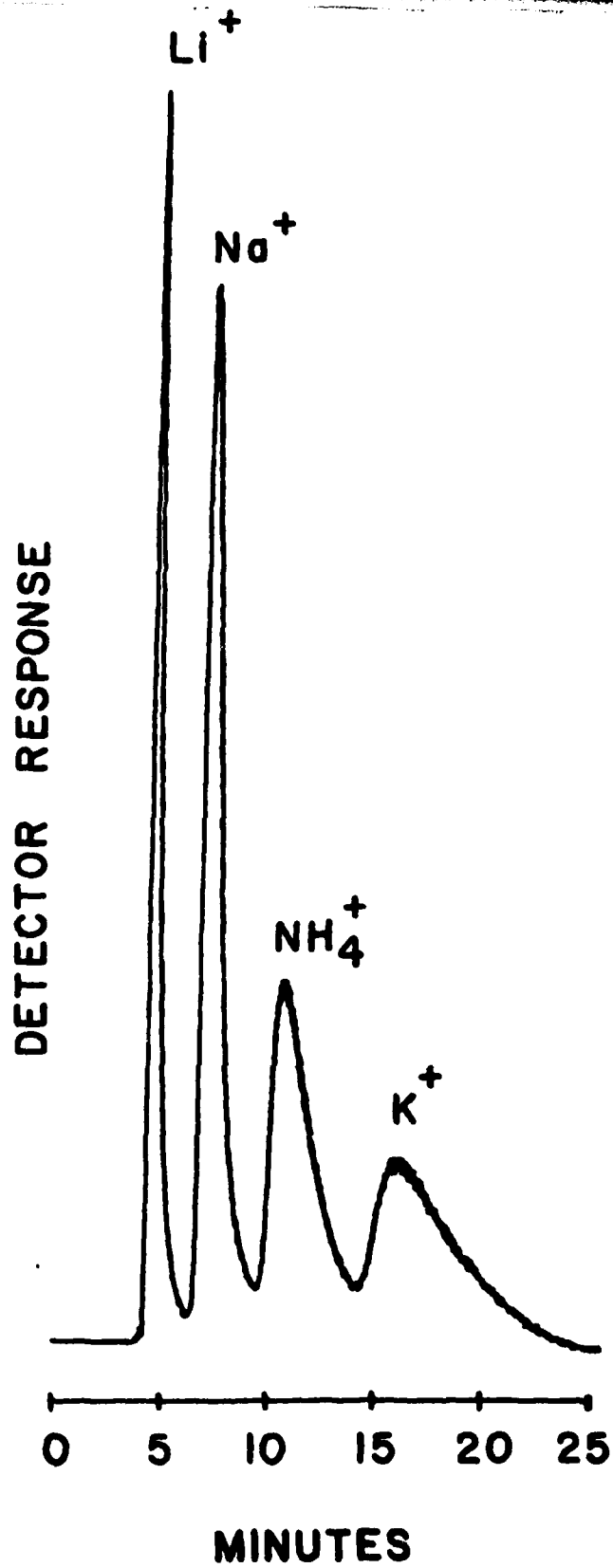
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